Handling Fluids in Microsensors

***Wanted:** Small, portable devices for automatically detecting and identifying viruses, bacteria, and toxic chemicals.*

***Reward:** Satisfaction in knowing that microsensors may help save lives.*

WORK has been under way for several years at sites throughout the departments of Energy and Defense on autonomous devices for detecting biological and chemical agents. The goal is to install them in subways, major office complexes, convention centers, or other sites where the public is at high risk of exposure to a covert release of biological or chemical agents. They will also be part of a network of sensors that will monitor urban areas or large events such as inaugurations or the Olympics. They will find their way onto the battlefield to protect soldiers in action. Used to analyze blood or other samples, such systems may detect and diagnose diseases in the field, far from laboratories and hospitals.

These monitoring systems must be robust and easy to operate and maintain. They must also have low power requirements to be truly portable and not rely on the large batteries that frequently accompany so-called field-portable devices today. And of course, the systems, like the tiny components that make them up, must be small and lightweight, which would not be possible without the ongoing revolution in microtechnology, particularly microelectromechanical systems. (See "The Microtechnology Center: When Smaller Is Better," *S&TR*, July/August 1997, pp. 11–17, and "Countering the Bioweapons Threat," *S&TR*, June 1998, pp. 4–9.)

Handheld instruments will incorporate microchip devices to take in an air or fluid sample; filter out smoke, dust, and other contaminants; mix the desired particles with fluid reagents as needed; and pump the mixture to sensors at the other end to determine what pathogens or toxins, if any, are present. Micromachined from silicon, glass, plastics, and ceramics, the components will have channels 20 to 200 micrometers deep and up to a millimeter wide through which fluids will travel. (Keep in mind that a human hair is just about 50 micrometers in diameter.)

Complete microfluidic systems have been a dream for more than a decade. At Livermore's Center for Microtechnology, experts in biology, electronics, optics, and engineering are working together on several unique components that will make them a reality.

Two Projects, One Goal

Two microfluidic projects, one sponsored by the Department of Energy and the other by the Department of Defense, are currently under way at the Center for Microtechnology. The sponsorship is different but the goals for both projects are to develop systems for handling fluids in autonomous detectors for biological pathogens.

Robin Miles leads the engineering team that is developing fluidic systems for DOE. This project, which includes participants from several DOE sites, is developing an autonomous device dubbed Sentry, whose functions will include continuous or on-demand air sampling, sample preparation, automated fluidic sample handling and transport, detection and identification of pathogens by immunoassay and DNA recognition, and automated data analysis and reporting.

Engineer Peter Krulevitch and his team are working under a subcontract to researchers at the University of Texas M. D. Anderson Cancer Center on the DoD project, which is sponsored by the Defense Advanced Research Projects Agency (DARPA) under the Microfluidic Molecular System (MicroFLUMES) Program. The purpose of this project is to develop an instrument that incorporates new technologies for separating particles (known as fractionation), sensing them, and identifying them based on their dielectric properties. Its first use will be to perform differential cell analysis on blood samples.

Building sophisticated, multifunctional, automated sample preparation systems for field use is still primarily a research and development activity. Most systems available commercially either assume a laboratory setting for testing or are designed for one use only. Also, many fractionation methods require filters that become clogged over time and contribute to the carryover of particles between tests. Furthermore, most operate using pneumatic power, which is excellent for microfluidic actuation because it can provide large forces over long distances and conform to the tiny tubes. But such systems require carting around a bulky canister of compressed air, and pneumatic valves cannot be miniaturized sufficiently to make these systems easily portable.

Both Livermore projects circumvent these challenges by using new methods of pumping, fractionation, mixing, and sample concentration and purification. Krulevitch's team has also developed a new sensing device that uses changes in impedance to identify particles.

The teams are working toward integrating their assorted components into a single instrument. Fabrication techniques, fluid conductivities, and fluid velocities, among other concerns, must be compatible for each overall system to perform optimally. System integration is key for both programs.

Inside Sentry

Because Sentry is intended for detecting and identifying biological or chemical warfare agents in the field, it is being designed to collect samples from the air. But samples could just as easily be blood or tissue for diagnosing disease. Processing the samples involves mixing reagents or microscopic polystyrene beads coated with antibodies with the potentially pathogenic particles. Pathogens in the sample will cling to beads coated with the appropriate antibody. Then the sample will be concentrated to facilitate the use of DNA-based assays. Combining a DNA-based assay with an antibody-based assay greatly increases Sentry's overall reliability in identifying pathogens.

Livermore engineer Amy Wang is exploring the use of acoustic energy to manipulate particles in the sample and to enhance the mixing of sample and reagent. Microscale mixing is a challenge because small channel dimensions make it difficult to create turbulence. Acoustic mixing brings with it the advantage of rapid mixing, no moving parts, and no need for nozzles or external injection of fluids to create turbulence. The ability to mix samples on the chip will speed up the rate of binding for immunoassays, increasing the throughput and speed of the system.

Electronic Filtering

Miles is using dielectrophoresis as a method of filtering the sample to collect the particles of interest in Sentry. Dielectrophoresis is an electrical phenomenon that allows particles to be trapped or manipulated by applying nonuniform electrical fields, which

induce electrical polarization in the particles. Depending on the polarizability of the particle with respect to the medium in which it is suspended, it will move toward or away from regions of high field intensity. Motion toward the regions of high field intensity is termed positive dielectrophoresis, while motion away from them is negative.

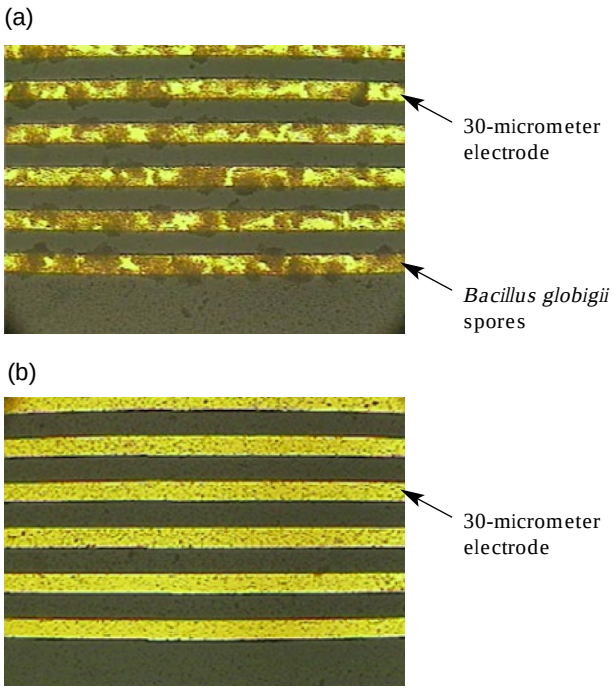
Dielectrophoretic forces provide an electrically switchable means to discriminate between particles and to manipulate them according to their dielectric properties. This phenomenon is ideally suited to microfluidic situations because large field strengths and correspondingly high dielectrophoretic forces are readily achievable with electrodes spaced less than a millimeter apart. Spores, bacteria, and cells, whose sizes range from 1 to 10 micrometers, may be captured with dielectrophoretic electrodes using less than 2 volts. Furthermore, dielectrophoretic forces are effective even for extremely small particles such as DNA.

Miles notes that this use of positive dielectrophoretic forces to electronically filter a sample—which has never been done commercially before—solves a couple of problems. First, it can be used to remove soil, smoke, pollen, mold spores, oil, and other contaminants from the raw sample. These contaminants may inhibit the operation of assays for DNA recognition based on the polymerase chain reaction (PCR), the most reliable method of identifying biowarfare organisms. Contaminants make antibody-based assays operate less effectively, too. Antibody assays are less specific than PCR, but they can be faster when the target organism is present in high concentrations. These assays also allow for simultaneous detection and identification of multiple biowarfare agents including toxins. Finally, using an electronic method to filter the sample eliminates the manual handling of samples and the large volumes of reagents and filter media typically used in laboratory analyses.

The dielectric properties of cells have been characterized by numerous experiments. But the dielectrophoretic properties of antibody-coated beads, spores, and DNA are less well known. Miles and her team have explored them in numerous experiments.

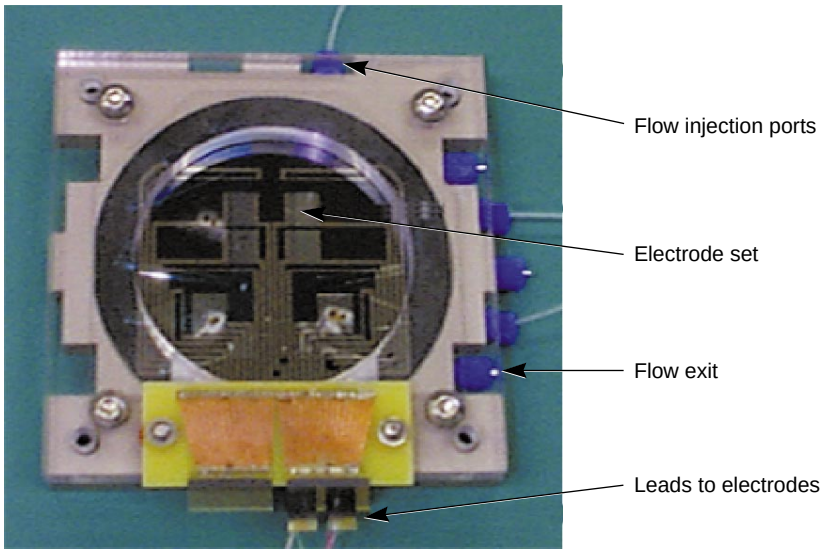
They studied the nontoxic *Bacillus globigii* spores and the vegetative bacteria *Erwinia herbicola*—simulants for anthrax and plague, respectively—to demonstrate their capture using dielectrophoretic electrodes. As shown at left, the force of attraction to the electrodes is sufficient to overcome the force on the spores due to fluid flow, allowing debris in the carrier fluid to be washed away while the spores are held in place. Another series of experiments determined the optimal capture frequency for several bioparticles of interest, including various types of DNA, *Bacillus globigii*, *Erwinia herbicola*, and beads.

On the basis of these and other experiments, the team believes it knows the best way to capture particles. Three parameters—the magnitude and direction of the dielectrophoretic force, the frequency of the electrodes, and the electrode geometry—are adjusted to selectively capture cells, spores, polystyrene beads, or DNA. Once the particles are captured, they are held in place against the flow of fresh carrier solution for a short time. Then the electric field is removed so the particles can be suspended in clean solution.



(a) *Bacillus globigii*, a simulant for anthrax, collects on activated electrodes as it flows through water. (b) The spores do not collect on inactivated electrodes.

The figure at top right shows the existing prototype of the component for testing the dielectrophoretic concentration of particles suspended in water. Other microfluidic pieces of Sentry are being designed to fit together with this component in an integrated system.



Component for testing the dielectrophoretic concentration of particles suspended in water. Acoustic subsystems and magnetohydrodynamic pumps for Sentry are being designed to integrate with it.

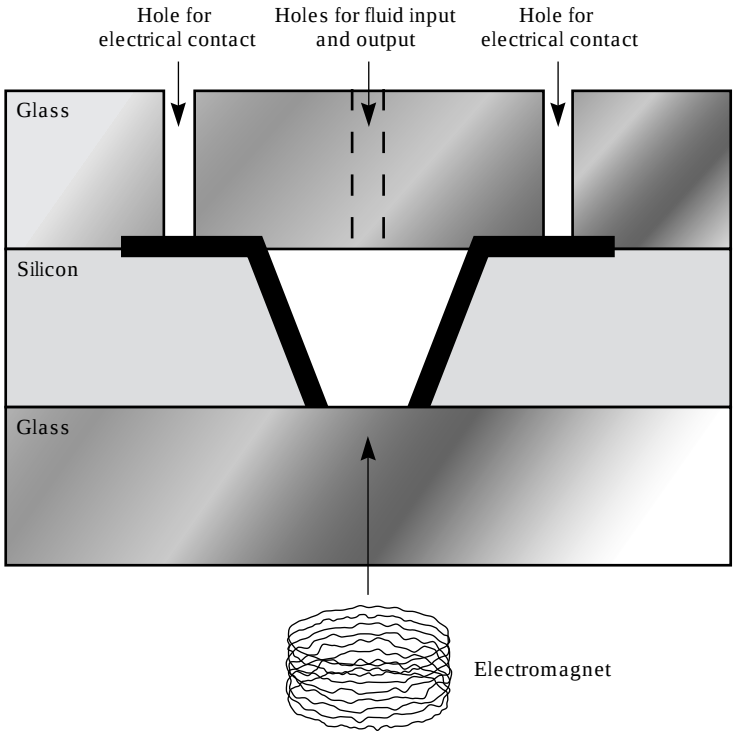
Keeping It Moving

Magnetohydrodynamic (MHD) pumps are being developed by physicist Sony Lemoff and others at Livermore to move fluid through all phases of Sentry's microfluidic system. They have been the first to demonstrate MHD pumps for aqueous solutions that function on a microchip and are pioneering the use of MHD pumps for micromachined applications.

There are several types of nonmechanical pumps, but thus far, the MHD pump is the most effective for producing a continuous, nonpulsating flow in a complex microchannel design. An MHD pump consists primarily of an electromagnet and a series of metal electrodes. Multiple pumps on the same chip can be driven independently by varying their electrode current amplitude and phase relative to the electromagnet, thus enabling routing in complex integrated microfluidic systems.

As shown in the figure at the lower right, the channels are etched through a silicon wafer with electrodes deposited on the walls of the channels. The silicon is then sandwiched between glass plates with holes for electrical contacts and fluid input and output. An electromagnet is positioned beneath the device.

The first-generation MHD pump had channels 380 micrometers deep and 800 micrometers wide, huge in the microtechnology world. Lemoff notes, "We are aiming for channels that are 20 by 50 or 50 by 100 micrometers. But



A cross section of the magnetohydrodynamic micropump. It is currently fabricated of glass and silicon, but the use of other materials is being explored.

with such small cross-sectional areas, we need higher magnetic field strength to push the fluid through." The early system used 0.025-tesla magnets, but they are being replaced with much more powerful 0.2-tesla ones.

Another Approach

The project with the University of Texas M. D. Anderson Cancer Center takes a different tack. Krulevitch and his team are responsible for microfabrication on the project and have designed the microfluidic chip.

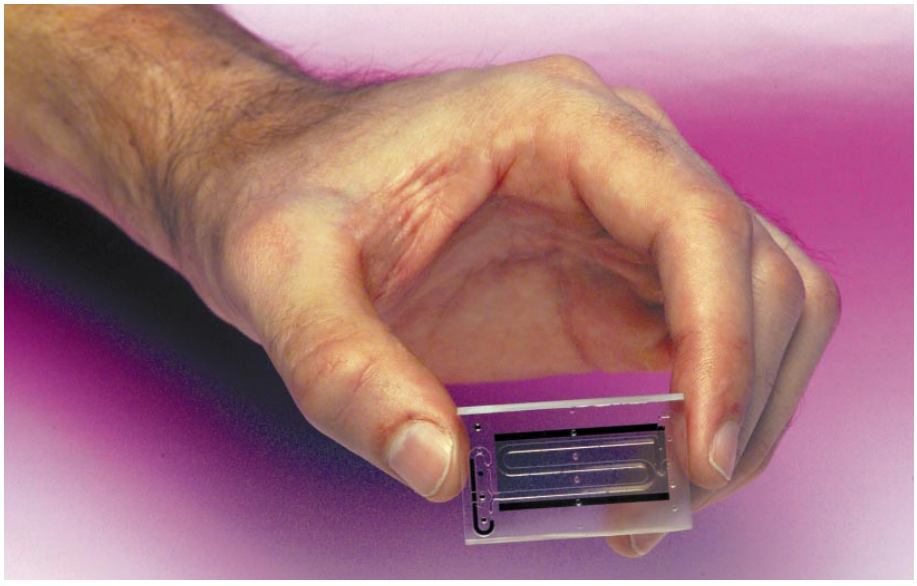
For fractionation, the DARPA project team is using negative dielectrophoresis to keep particles "levitated" in the microfluidic channel away from electrodes. Krulevitch's team together with the Anderson researchers have designed the microchannels and dielectrophoretic electrode arrays that make this separation system work.

The DARPA project team has also developed an impedance sensor that can detect particles as they leave the system and characterize them both by their time of passage through the separation column and by their size and dielectric frequency spectrum. This sensor consists of two electrodes on opposite sides of the channel. The resistance and capacitance—and hence the impedance—between the two electrodes is already known. When a particle passes between the electrodes, the impedance value changes. Direct-current impedance sensors are used in commercial blood cell and microparticle counters, but they indicate only particle size. This new version, using alternating current, represents a considerable improvement.

The integrated injection—separation—detection component shown below consists of microscopic sorting channels 150 micrometers deep, 1 millimeter wide, and 10 centimeters long arranged in serpentine fashion on a 2.5- by 4-centimeter substrate. Channels are equipped with integrated arrays of dielectrophoretic electrodes 50 micrometers wide with 50-micrometer-wide gaps. At the exit of the channel are impedance sensors with their independent electrodes.

In operation, a micropump sends 1-microliter samples of 10-micrometer-diameter surface-coated beads (for detecting warfare agents) or human cells (for detecting disease) into the microchannel. The samples are then slowly flushed through with fluid from another small reservoir. Beads or cells are detected as they pass the impedance sensors after fractionation along the channel.

At the M. D. Anderson Cancer Center, both the fractionation process and the impedance sensor using both beads and cells have performed well in initial tests. Plans call for the fully integrated blood-analysis fractionation system, which is being packaged by LYNNTECH, Inc., of College Station, Texas, to be delivered to DARPA in the fall of 2000.



The integrated injection–separation–detection system being developed by the DARPA project team resides on a 2.5- by 4-centimeter substrate.

Simulations of Microactivity

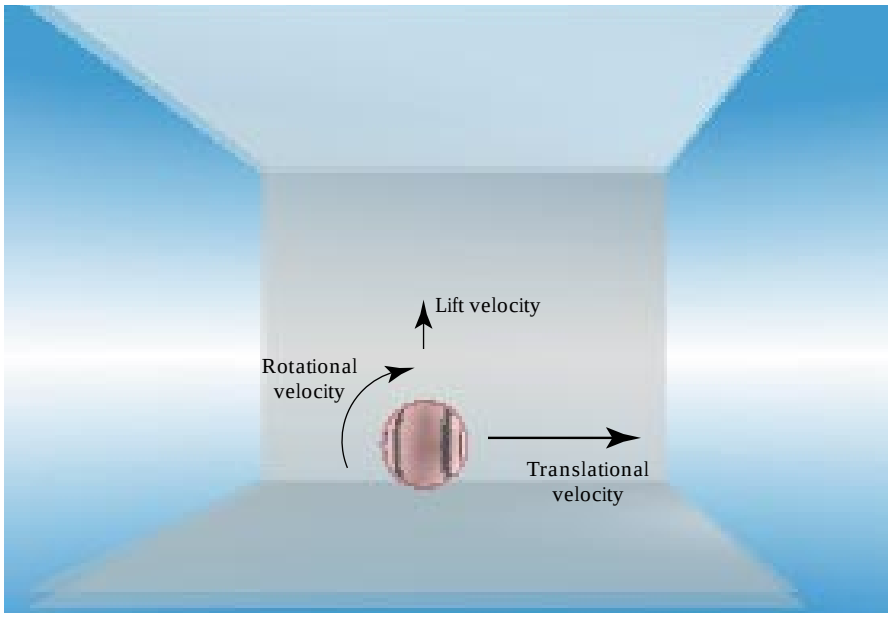
The transport and manipulation of beads and pathogenic particles must be predictable for all of the components to operate together in the DOE and DARPA systems.

To guide work on these subsystems, engineer David Clague has developed an enhanced three-dimensional, discrete simulation model that permits the study of stationary and mobile particles in microfluidic devices. The model is being extended to incorporate intermolecular force interactions. Because the channels in microfluidic systems are so small, intermolecular forces, which are typically masked in laboratory-scale instruments, affect the behavior of particles as they move through the system and are manipulated.

Known as lattice Boltzmann, the model is based on the Boltzmann

equation, which easily incorporates external forces, thermal diffusion, and device wall interactions in studies of the dynamic behavior of a collection of particles (see the figure at right). This contrasts with traditional microfluidic modeling based on finite-element analysis and boundary-element methods, which in this area of research typically deal only with pure fluid effects.

In the near future, Clague will incorporate electromagnetic-induced pressure fields to study particle behavior during each process in the two instruments. As work on the components progresses and the configuration of the overall systems is determined, simulations will help explain the interplay of fundamental-force interactions in each subsystem.



A lattice Boltzmann model of fluid flow and particle movement in a microchannel. In this cutaway side view of a microchannel, the sphere representing a particle moves from left to right. Inertial forces and wall interactions tend to lift the rotating sphere, translating it toward the center of the microchannel. The DARPA project team is attempting to exploit this lift phenomenon in the design of a fractionation device based on negative dielectrophoresis.

Fabricating the Systems

The goal of all this work is to have fully integrated systems. Nowhere does this present a greater challenge than in the area of fabrication. Currently, no single-material system has proven superior to all others. Each has its merits and drawbacks, depending on the specific function of the component. When the various functions are being integrated, multiple-material systems must be considered, including associated packaging and interconnection technologies.

The most commonly used materials for microfluidic systems are polymers, silicon, and Pyrex glass. Livermore has also begun fabricating microfluidic devices using ceramics and other glasses. Engineer Harold Ackler is developing fabrication processes for both projects and exploring a number of fabrication methods for the various subsystems.

He and other Livermore researchers are developing proprietary technology that will integrate new ceramic and glass devices with commercially available microelectronic packaging and other proprietary Livermore fluidic interconnect technology. This integrative capability will make the now troublesome task of making fluidic and electronic connections as simple as plugging in a packaged integrated circuit. Ackler notes, "Being able to integrate these devices so easily is extremely attractive for systems deployed in the field in which replacement of a component or a consumable material like a reagent must be quick and simple."

The team has several material-related issues to handle. Eliminating the most rapid corrosion mechanism has solved the problem of electrode corrosion. Problems with spores, dirt, DNA, and other materials adhering to the various surfaces in the system are being dealt with. The first-generation MHD pump was made of silicon and Pyrex glass, but newer ceramic and glass alternatives are also being studied.

While it may be possible to fabricate all components with the same materials, some functions may not be optimal. So Livermore is taking a dual approach, examining the use of discrete components as well as pursuing the development of a fully integrated fabrication method wherein all components are integrated into the same piece of material. The latter approach should reduce system size, power requirements, use of consumables, packaging problems, and manufacturing costs. But if the use of discrete components results in superior system performance, Ackler will take that route, making use of Livermore's integration and packaging technology.

Complete Systems Soon

The Center for Microtechnology is involved in numerous microfluidic projects. In addition to the microfluidics

work for DOE and DARPA, the center is participating with the University of Minnesota and three other universities in a proposal submitted in recently to the National Science Foundation for a Center for Biomedical Microsystems. If the proposal is funded, 22 industrial partners have committed to assist the collaboration in developing diagnostic and therapeutic microsystems, including micropumps and other microfluidic subsystems.

For work under way now, a fully integrated microfluidic system for the DARPA project will be delivered next year, while completion of the DOE detector is about three years away. Ray Mariella, director of Livermore's Center for Microtechnology, is pleased with successes to date. "Livermore has been a leader in the microfabrication world for quite a while. Producing a usable microfluidic system as part of a detector for biological and chemical agents will be a real feather in our cap."

—Katie Walter

Key Words: Center for Microtechnology, dielectrophoresis, impedance sensor, magnetohydrodynamic (MHD) pump, microdevices, microfluidics.

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About the Engineers

ROBIN MILES joined Lawrence Livermore in 1997 as a mechanical engineer specializing in the development of microdevices for biological applications. Currently, she is an engineer and group leader in the Laboratory's Center for Microtechnology. She is coprincipal investigator for sample preparation on an autonomous, continuous monitoring system for counterbiological warfare pathogens and principal investigator on a project to build a biological processor using electric fields.

Miles earned an B.S. in mechanical engineering from the Massachusetts Institute of Technology, an M.S. in mechanical engineering from Stanford University, and an M.B.A. from the University of California at Berkeley.

PETER KRULEVITCH holds a B.S., M.S., and Ph.D. from the University of California—all in mechanical engineering. He joined Lawrence Livermore in 1994 as a postdoctoral fellow and is currently a microelectromechanical systems researcher in the Engineering Directorate's Center for Microtechnology. He is principal investigator for a project to develop microfabricated cell separation and detection systems in collaboration with the University of Texas M. D. Anderson Cancer Center. He is also working on projects to develop shape-memory film microactuators for medical and microfluidic applications, to create finite-element models of micromechanical devices, to microfabricate a temperature–pH biosensor, and to investigate the mechanical properties of thin films for multilayer mirrors.

Krulevitch is the coholder of eight U.S. patents for a variety of microdevices and their fabrication methods, primarily for medical and biotechnology applications.